United States Department of Agriculture
Animal and Plant Health Inspection Service
Plant Protection and Quarantine

Using ELISA as part of a *Phytophthora ramorum* Nursery Survey

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Introduction:

The Washington State Department of Agriculture has successfully used a direct sandwich enzyme linked immunosorbent assay (DAS ELISA) as a primary screening tool in our *Phytophthora ramorum* nursery survey. This method offers four principal advantages; 1) speed – 96 test wells can comfortably be processed by a single person in a two-day period 2) simplicity – nearly anyone can be trained to carry out the ELISA procedure and very little specialized equipment is necessary 3) efficiency - samples can be bulked for processing and 4) cost – per-sample screening costs are low, especially if samples are bulked and entire plates (96 wells) are run at a given time. Approximately 95% of our samples test negative with the ELISA screen, eliminating the need for further assay. Samples testing positive by ELISA are plated on PARP.

ELISA Details:

We use the "DAS ELISA test system for Phytophthora" developed by *Agdia Inc.*, Elkhart, IN. This is a generalized test for the genus *Phytophthora* and is geared towards running multi-well plates (it is not a single test "kit"). Test details, protocols & materials costs can all be easily accessed on Agdia's web site at www.agdia.com. Testing protocols can be found at www.agdia.com/cgi_bin/catalog.cgi?m39.

Selecting plant material for the Assay:

We use the same criteria for selecting tissue for the ELISA screen that is used to select tissue for plating on PARP. The ratio of leaf tissue to buffer is defined in the ELISA protocol (1:10). We usually test 5 leaves in a single bulk sample (all from the same variety-block... NEVER bulk across blocks) one tissue piece per leaf. Subsequent "PARPing" of ELISA positives has yielded as few as 1 *P. ramorum* grow-out per plate ... so it appears that the ELISA sensitivity is adequate for the demands we place on it (1 in 5). We usually take 5 sections of tissue from 3 - 5 mm square. Tissue is taken from the margins of lesions, midrib, petiole, leaf tip, etc. as necessary. Symptomatic tissue is always preferable; if none is available we go for the most likely infection sites (leaf tip and petiole). Amount of buffer for grinding ranges from 800 – 1000, depending on volume of tissue and how desiccated it is. We use Agdia's ball-bearing grinder mounted to a drill press to process samples for ELISA. Sample is ground until buffer/sap changes color to a bright green or nearest equivalent. Usually just a few seconds is required when the ball bearing grinder is mounted on the drill press (contact Agdia for details 1-800-622-4342).

Reading the plates:

While an automated plate reader is nice, in our experience it is unnecessary. All of the samples in our survey that have yielded *Phytophthora* cultures have given a distinct, visual result on the ELISA plate. The reader provides useful and impartial documentation of the assay results, but does not in our opinion improve our ability to detect *Phytophthora spp.* in plant tissue.

Cost:

Using the ELISA screen is very cost effective, especially if you process enough samples to run full 96-well plates and even more so if you bulk your samples (five samples per well). What follows is chart of the costs of all perishable goods associated with the ELISA assay including reagents, buffers, controls, plates, and grinding bags. Prices are taken directly from the *Agdia Inc.* web site as of 12/9/03.

Perishable Goods

Total # of test wells	Total cost	Cost per well	Cost per sample (5/well)
500	$500.00	$1.00	$0.20
1000	$829.00	$0.82	$0.17
5000	$3103.00	$0.62	$0.12

Labor Costs

Obviously this will vary greatly. We estimate a single person will spend approximately 11 hours in a two-day period processing a single 96-well plate. At our current rate of compensation, this adds approximately $1.60 per well, or $0.32 per sample to the cost of the assay.

One-time and/or Infrequent Expenditures

The lab will need certain basic equipment: refrigerator, 8-channel micropipette, 0.1 ml micropipette, pipette tips, wash bottles, reagent wells, and enough basic lab equipment to formulate reagents & buffers. Excepting the refrigerator, all other equipment can be purchased for well under $1,000.00.

Improving Efficiency:

- Have multiple people involved in the cutting and grinding part of the procedure, once this is done, a single person can finish processing several plates on their own.
- We make our own buffers from scratch each week. It's cheaper (materials-wise) and we don't have to use sodium azide as a preservative (which is highly toxic and difficult to dispose of).
- We grind our samples using a drill press with a special grinding attachment ($75 from Agdia). It speeds the process and removes the physical limitation on the number of samples a single person can grind in a day. Using manual methods (hammer and board) most folks' arms give out after a single plate.
- Shop around for the 96-well plates, you can often find them at significantly reduced prices.

Summary:

DAS ELISA has proven to be an effective way of screening plant tissue for the presence of *Phytophthora spp*. for Washington's *P. ramorum* survey. We estimate that we spend less than $0.50 per sample on our assay procedure. This is a simple and fast screening technique well suited to small laboratories with limited resources. It does not take the place of plating on PARP or PCR analysis in determining the presence of *P. ramorum*, but it drastically reduces the number of samples on which these more expensive and time consuming procedures need to be performed.

References

Additional information on culturing and identification:

1. http://www.cnr.berkeley.edu/comtf/
This site is part of the Sudden Oak Death and Oak Mortality Task Force in California. It contains links to a great deal of information on various aspects of *P. ramorum* and the diseases it causes.

2. http://cropandsoil.oregonstate.edu/people/faculty.php?ID=39
This site (Jennifer Parke – OSU) includes a PDF of a color pamphlet entitled: *"Phytophthora ramorum*: a guide for Oregon nurseries." This site also includes a link to excellent images of chlamydospores and sporangia.

3. Davidson, et al. 7 July 2003. Plant Management Network (http://www.plantmanagementnetwork.org/).

4. Werres, S., Marwitz, R., Man Int' Veld, W.A., De Cock, A.W.A.M., Bonants, P.J.M., De Weerdt, M., Themann, K., Ilieva, E., and Baayen, R.P. 2001. *Phytophthora ramorum* sp. nov., a new pathogen on *Rhododendron* and *Viburnum*. Mycological Research 105:1155-1165.
This site is the original description of *P. ramorum*, and a comparison with other similar species.

Also please note the California Oak Mortality Task Force website (http://www.suddenoakdeath.org). This site has numerous pictures of many of the regulated and associated hosts with symptoms caused by *P. ramorum*.